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Patentanmeldung Nr.	Patent application No.	Demande de brevet n°
	99870068.6	

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

DEN HAAG, DEN
THE HAGUE, 30/07/03
LA HAYE, LE

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Method for the amplification of exon 2 and exon 3 of HLA class I alleles

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METHOD FOR THE AMPLIFICATION OF EXON 2 AND EXON 3 OF HLA CLASS I ALLELES

Field of the invention

The present invention relates to a method for the typing or subtyping of HLA-A, HLA-B or HLA-C. More specifically, the present invention relates to a method for the locus-specific, separate amplification of exon 2 and/or exon 3 of HLA-A, HLA-B or HLA-C alleles.

Background of the invention

The human major histocompatibility complex (MHC) is contained within about 4 Mbp of DNA on the short arm of chromosome 6 at 6p21.3 (Campbell and Trowsdale, 1993). The human MHC is divided into class I, class II and class III regions. The genes of class I and class II encode highly polymorphic cell-surface molecules that bind and present processed antigens in the form of peptides to T-lymphocytes, initiating both cellular and humoral immune responses. The class I molecules, HLA-A, -B, and -C, are found on most nucleated cells. They are cell-surface glycoproteins that bind and present processed peptides derived from endogenously synthesized proteins to CD8+ T-cells. These heterodimers consist of an HLA-encoded α-chain associated with a non-MHC encoded monomorphic polypeptide, β_2-microglobulin (Townsend and Bodmer, 1989; Spencer and Parham, 1996). The class II molecules

are encoded in the HLA-D region. These cell-surface glycoproteins consist of HLA-encoded α-, and β-chains, associated as heterodimers on the cell surface of antigen-presenting cells such as B-cells and macrophages. Class II molecules serve as receptors for processed peptides. However, these peptides are derived predominantly from membrane and extracellular proteins and are presented to CD4+ T-cells. The HLA-D region contains several class II genes and has three main subregions: HLA-DR, -DQ, and -DP. Both the HLA-DQ and -DP regions contain one functional gene for each of their α- and β-chains. The HLA-DR subregion contains one functional gene for the α-chain; the number of functional genes for the β-chain varies from one to two according to the haplotype (Andersson et al., 1987; Apple and Erlich, 1996).

Extensive polymorphism exists at most loci. In view of the biological and medical importance of these antigens, a highly sensitive and rapid technique for HLA typing is required. A variety of techniques are currently used to detect HLA polymorphism, including serological, biochemical, T-cell recognition and, most recently, molecular biological methods.

Serology remains the mainstay method for HLA typing - especially for class I - for many routine histocompatibility laboratories. The micro-lymphocytotoxicity assay (Kissmeyer et al., 1969; Terasaki and McClelland, 1964) is the standard approach: viable peripheral blood mononuclear cells (class I) or separate B-cells (class II) are mixed with antisera (polyclonal or monoclonal) of known HLA specificity.

Detection of polymorphism can be achieved by looking at the different amino acid composition of HLA molecules through biochemical techniques such as one-dimensional isoelectric focusing (IEF; Yang, 1987). This method relies on amino acid substitutions contributing to changes in charge of the HLA molecule.

Another HLA typing method is the mixed lymphocyte reaction (MLR). Concurrent to observations being made using HLA-specific antisera, it was noted that lymphocytes from two unrelated sources, when mixed in culture, would proliferate (Hirschorn et al., 1963).

Analysis of HLA specificities from DNA provided a new approach to defining their polymorphic differences. Rather than looking at differences in the expressed molecule, polymorphism is characterized at the nucleotide level.

An important and powerful development in the field of molecular biology has been the polymerase chain reaction (PCR; Mullis et al., 1986; Mullis and Faloona, 1987). In tissue typing, PCR is used to amplify the polymorphic regions of HLA genes. This HLA PCR product can then be analysed for its polymorphic differences, to establish the tissue type. A number of such approaches have been developed, including hetero duplex analysis of PCR products (Clay et al., 1994), single-stranded conformational polymorphism analysis of the PCR product (PCR-SSCP; Yoshida *et al.*, 1992), sequence-based typing (SBT; Santamaria et al., 1992 and 1993), the use of sequence specific primers in PCR reaction (PCR-SSP; Olerup and Zetterquist, 1991), the use of PCR in combination with sequence-specific oligonucleotide probing (PCR-SSOP; Saiki et al., 1986) or probing by reverse dot-blot (Saiki et al., 1989). These approaches, used singly or in combination, have all been applied as DNA-based methods for tissue-typing of class I and class II HLA specificities. DNA typing methods should be preferred over serological methods provided that an easy, rapid and reliable DNA typing method is available. Some differences at the subtype level which are detectable by DNA methods might go undetected by current serological typing methods, although these differences might provoke allograft rejection (Fleischhauer et al., 1990).

The HLA system is the most polymorphic human genetic system yet known. The HLA class I alleles contain polymorphic substitutions which are mostly located in both exon 2 and exon 3, encoding the peptide binding groove of the class I molecule. These polymorphisms make differentiation between alleles achievable through a variety of molecular biological techniques such as sequencing or hybridization with relevant probes. In the current diagnostic kits exon 2 and exon 3 are amplified together, resulting in amplicons of about 1 kb, consisting at least of exon 2, intron 2 and exon 3. Locus-specific primers are available for the amplification of these 1 kb amplicons. However, such large amplicons are difficult to amplify and show secundary structure formation resulting in inefficient hybridization of some probes. Therefore, a separate amplification of exon 2 and/or exon 3 would be desired which enables a more efficient typing of HLA class I alleles. However, as locus-specific primer annealing sites are not present in exon 2 or exon 3, separate and locus-specific amplification of exon 2 and/or exon 3 of HLA-A, HLA-B or HLA-C is not evident.

Aims of the invention

It is an aim of the present invention to provide a method for the locus-specific and separate amplification of exon 2 and/or exon 3 of HLA-A, HLA-B or HLA-C alleles.

It is a more specific aim of the present invention to provide a method for a one-step, locus-specific, separate amplification of both exon 2 and exon 3 of HLA-A, HLA-B or HLA-C alleles.

It is another aim of the present invention to provide an improved method for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample.

It is another aim of the present invention to provide a primer for use in a method for the locus-specific, separate amplification of exon 2 of HLA-A, HLA-B or HLA-C alleles.

It is another aim of the present invention to provide a primer for use in a method for the locus-specific, separate amplification of exon 3 of HLA-A, HLA-B or HLA-C alleles.

It is a more specific aim of the present invention to provide a primer set for use in a method for the amplification of exon 2 of HLA-A, HLA-B or HLA-C alleles.

It is another more specific aim of the present invention to provide a primer set for use in a method for the amplification of exon 3 of HLA-A, HLA-B or HLA-C alleles.

It is another more specific aim of the present invention to provide a multiplex primer mix for a one step, locus-specific, separate amplification of exon 2 and exon 3 of HLA-A, HLA-B or HLA-C.

It is another aim of the present invention to provide an improved diagnostic kit for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample.

It is a more specific aim of the present invention to provide an improved line probe assay for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample.

Detailed description of the invention

The present invention relates to a method for the locus-specific, separate amplification of exon 2 and/or exon 3 of HLA-A, HLA-B or HLA-C alleles, making use of at least one primer set wherein:

- for the amplification of exon 2, the reverse primer specifically hybridizes to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C;
- for the amplification of exon 3, the forward primer specifically hybridizes to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C.

The amplification method of the invention thus, makes use of a primer set of which one of the primers is hybridizing to a target sequence in intron 2 in order to amplify only exon 2 or only exon 3. In the case where exon 2 is amplified, the reverse primer hybridizes to a locus-specific target sequence in intron 2 while the forward primer might be chosen to hybridize to a region upstream of exon 2 (e.g. in intron 2 or in exon 1) or to the initial nucleotides of exon 2. In the case where exon 3 is amplified, the forward primer is hybridizing to a locus-specific target sequence in intron 2 while the reverse primer might be chosen to hybridize to the final nucleotides of exon 3 or downstream of exon 3 (e.g. in intron 3). This new amplification method will result in the amplification of shorter DNA fragments, containing only exon 2 or only exon 3, which are much easier to amplify and much easier for use in different typing methods such as hybridization with different allele specific probes or sequencing. From the example section it is clear that primer sets containing one primer hybridizing to a

target sequence in intron 2 provide a much better and easier amplification of exon 2 and exon 3 of HLA Class I alleles.

The term "primer" refers to a single stranded oligonucleotide sequence capable of acting as a point of initiation for synthesis of a primer extension product that is complementary to the nucleic acid strand to be copied. The length and the sequence of the primer must be such that they allow to prime the synthesis of the extension products. Preferably, the length of the primer is about 5-50 nucleotides. More preferably, the length of the primer is about 10-30 nucleotides. Most preferably, the length of the primers is about 20-25 nucleotides. Specific length and sequence will depend on the complexity of the required DNA or RNA target, as well as on the conditions at which the primer is used, such as temperature and ionic strength.

The expression "primer set" refers to a pair of primers allowing the amplification of part or all of exon 2 or exon 3 of HLA-A, HLA-B or HLA-C. A primer set always consists of a forward primer (or 5' primer) and a reverse primer (or 3' primer).

The term "to hybridize specifically" means that, during the amplification step, said primer forms a duplex with part of its target sequence or with the entire target sequence under the experimental conditions used, and that under those conditions said primer does not form a duplex with other sequences of the polynucleic acids present in the sample to be analysed. It should be understood that primers that are designed to specifically hybridize to a target sequence of a nucleic acid, may fall within said target sequence or may to a large extent overlap with said target sequence (i.e. form a duplex with nucleotides outside as well as within said target sequence).

The term "target sequence" of a primer according to the present invention is a sequence within intron 2 of the HLA Class I alleles to which the primer is completely complementary or partially complementary (i.e. with up to 20%, 15%, 10% or 5%

mismatches). It is to be understood that the complement of said target sequence is also a suitable target sequence in some cases. The fact that amplification primers do not have to match exactly with the corresponding target sequence in the template to warrant proper amplification is amply documented in the literature (Kwok et al., 1990). However, when the primers are not completely complementary to their target sequence, it should be taken into account that the amplified fragments will have the sequence of the primers and not of the target sequence.

In one embodiment, the amplification is performed in a reaction tube with a primer set for the amplification of exon 2 as described above (reverse primer hybridizes with a target sequence in intron 2) and consequently only exon 2 is amplified.

In another embodiment, the amplification is performed in a reaction tube with a primer set for the amplification of exon 3 as described above (forward primer hybridizes with a target sequence in intron 2) and consequently only exon 3 is amplified. Accordingly, the present invention relates to a method for the separate amplification of exon 2 or exon 3 of HLA Class I alleles.

In a preferred embodiment, the different primers sets involved in the amplification of exon 2 and the amplification of exon 3 are mixed and the separate amplification of both exon 2 and exon 3 is performed in a single reaction tube. Thus, the present invention also relates to a method as described above further characterized that both exon 2 and exon 3 of HLA-A, HLA-B or HLA-C are amplified by use of a multiplex primer mix containing at least one primer pair for amplification of exon 2 and at least one primer pair for amplification of exon 3. Accordingly, the present invention relates to a method for the separate amplification of exon 2 and exon 3 of HLA Class I alleles.

The primers are specifically selected in a way that the amplification of exon 2 and/or exon 3 of HLA-A, HLA-B or HLA-C is locus-specific. The term "locus-specific" means that exon 2 and/or exon 3 of only one locus (i.e. HLA-A, HLA-B or HLA-C) is amplified while exon 2 and/or exon 3 of the other 2 loci is not amplified. Thus, when primers specific for amplification of exon 2 and/or exon 3 of HLA-A are used, exon 2 and/or exon 3 of HLA-B and HLA-C are not amplified. Similarly, when primers specific for amplification of exon 2 and/or exon 3 of HLA-B are used, exon 2 and/or exon 3 of HLA-A and HLA-C are not amplified. Similarly, when primers specific for amplification of exon 2 and/or exon 3 of HLA-C are used, exon 2 and/or exon 3 of HLA-A and HLA-B are not amplified. From this it is clear that exon 2 and/or exon 3 of 2 or 3 different HLA loci can never be amplified together. Thus, exon 2 and/or exon 3 of HLA-A can never be amplified in the same reaction tube with exon 2 and/or exon 3 of HLA-B. Similarly, exon 2 and/or exon 3 of HLA-A can never be amplified in the same reaction tube with exon 2 and/or exon 3 of HLA-C. Similarly, exon 2 and/or exon 3 of HLA-B can never be amplified in the same reaction tube with exon 2 and/or exon 3 of HLA-C. Similarly, exon 2 and/or exon 3 of HLA-A can never be amplified in the same reaction tube with exon 2 and/or exon 3 of HLA-B and exon 2 and/or exon 3 of HLA-C. As a consequence, the present invention relates to a method for the locus-specific, separate amplification of exon 2 and/or exon 3 of HLA-A, HLA-B or HLA-C alleles. This means that the invention relates to a method for the separate amplification of exon 2 and/or exon 3 of HLA-A, the invention relates to a method for the separate amplification of exon 2 and/or exon 3 of HLA-B and the invention relates to a method for the separate amplification of exon 2 and/or exon 3 of HLA-C.

The amplification method used can be either polymerase chain reaction (PCR; Saiki et al., 1988), ligase chain reaction (LCR; Landgren et al., 1988; Wu & Wallace, 1989; Barany, 1991), nucleic acid sequence-based amplification (NASBA; Guatelli et al., 1990; Compton, 1991), transcription-based amplification system (TAS; Kwoh et al., 1989), strand displacement amplification (SDA; Duck, 1990) or amplification by means of Qß replicase (Lomeli et al., 1989) or any other suitable method to amplify nucleic acid molecules known in the art.

In a preferred embodiment, the present invention relates to a method as described above, further characterized that the locus-specific target sequences are situated at one of the following positions:

- 67, 96, 109, 110, 111, 118, 123, 131 or 181 of the HLA-A intron 2; or
- 35 or 170 of the HLA-B intron 2; or
- 96, 119 or 154 of the HLA-C intron 2.

As these positions all contain locus-specific nucleotides, these position are particularly suitable for designing an efficient primer for the locus-specific amplification of exon 2 or exon 3. The primers can be of different length. Preferably, the length of the primer is about 5-50 nucleotides. More preferably, the length of the primer is about 10-30 nucleotides. Most preferably, the length of the primers is about 20-25 nucleotides. Specific length and sequence will depend on the complexity of the required DNA or RNA target, as well as on the conditions at which the primer is used, such as temperature and ionic strength.

According to an even more preferred embodiment, the present invention relates to a method such as described above further characterized that said positions constitute the 3' end of the primer that is used for the amplification of exon 2 or exon 3. Thus, from the above described positions, forward as well as reverse primers can be designed that

have their 3' end in these specific positions. The forward primers having their 3' end in the above mentioned specific positions will enable amplification of exon 3 of the respective HLA-A, HLA-B or HLA-C alleles. The reverse primers having their 3' end in the above mentioned specific positions will enable amplification of exon 2 of the respective HLA-A, HLA-B or HLA-C alleles. The primers can be of different length. Preferably, the length of the primer is about 5-50 nucleotides. More preferably, the length of the primer is about 10-30 nucleotides. Most preferably, the length of the primers is about 20-25 nucleotides. Specific length and sequence will depend on the complexity of the required DNA or RNA target, as well as on the conditions at which the primer is used, such as temperature and ionic strength.

According to the most preferred embodiment, the present invention relates to a method as described above, further characterized that the primer is chosen from the following list:

- for the amplification of exon 2 of HLA-A (table 1):

5'ATCTCGGACCCGGAGACTGT3' (SEQ ID NO 1)
5'GATCTCGGACCCGGAGACTGT3' (SEQ ID NO 2)
5'GGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 3)
5'YGGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 4)
5'GYGGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 5)
5'GGYGGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 6)

5'GGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 7)
5'GGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 8)
5'AGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 9)
5'AAGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 10)
5'CAAGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 11)

5'CTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 12)
5'TCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 13)
5'CTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 14)

5'CCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 15)
5'GCCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 16)
5'GGCCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 17)

5'TCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 18)
5'CTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 19)
5'CCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 20)
5'GCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 21)
5'GGCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 22)
5'GGGCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 23)

5'CTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 24)
5'CCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 25)
5'GCCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 26)
5'GGCCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 27)
5'GGGCCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 28)
5'TGGGCCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 29)

5'CCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 30)
5'GCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 31)
5'CGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 32)
5'GCGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 33)
5'GGCGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 34)
5'AGGCGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 35)

5'AGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 36)
5'AAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 37)
5'WAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 38)
5'TWAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 39)
5'GTWAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 40)
5'GGTWAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 41)

5'CCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 42)
5'ACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 43)
5'AACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 44)
5'AAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 45)
5'GAAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 46)

5'TGAAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 47)

5'YCCVGCCCCGACCAACCYGG3' (SEQ ID NO 48)
5'GYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 49)
5'YGYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 50)
5'CYGYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 51)
5'CCYGYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 52)
5'CCCYGYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 53)

- for the amplification of exon 3 of HLA-A (table 2):

5'CGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 54)
5'ACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 55)
5'CACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 56)
5'CCACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 57)
5'CCCACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 58)
5'CCCCACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 59)

5'GGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 60)
5'GGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 61)
5'CGGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 62)
5'CCGGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 63)
5'TCCGGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 64)
5'CTCCGGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 65)

5'CCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 66)
5'RCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 67)
5'CRCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 68)
5'CCRCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 69)
5'TCCRCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 70)
5'ATCCRCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 71)

5'CCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 72)
5'CCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 73)
5'RCCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 74)
5'CRCCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 75)
5'CCRCCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 76)
5'TCCRCCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 77)

5'CCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 78)
5'CCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 79)
5'CCCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 80)
5'RCCCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 81)
5'CRCCCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 82)
5'CCRCCCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 83)

5'CGCGGGACYCCGAGACCCTT3' (SEQ ID NO 84)
5'CCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 85)
5'GCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 86)
5'AGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 87)
5'AAGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 88)
5'GAAGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 89)

5'GACYCCGAGACCCTTGDCCC3' (SEQ ID NO 90)
5'GGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 91)
5'GGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 92)
5'CGGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 93)
5'GCGGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 94)
5'CGCGGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 95)

5'GACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 96)
5'AGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 97)
5'GAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 98)
5'CGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 99)
5'CCGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 100)
5'YCCGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 101)

5'GTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 102)
5'AGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 103)
5'CAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 104)
5'TCAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 105)
5'TTCAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 106)
5'TTTCAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 107)

- for the amplification of exon 2 of HLA-B (table 3):

5'CCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 108)

5'ACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 109)
5'AACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 110)
5'CAACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 111)
5'CCAACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 112)
5'MCCAACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 113)

- for the amplification of exon 3 of HLA-B (table 4):

5'CYGGGGCGSAGGTCACGACT3' (SEQ ID NO 114)
5'CCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 115)
5'GCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 116)
5'GGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 117)
5'CGGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 118)
5'CCGGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 119)

5'CCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 120)
5'ACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 121)
5'TACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 122)
5'YTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 123)
5'TYTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 124)
5'STYTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 125)

- for the amplification of exon 2 of HLA-C (table 5):

5'GTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 126)
5'GGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 127)
5'CGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 128)
5'CCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 129)
5'YCCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 130)
5'CYCCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 131)

- for the amplification of exon 3 of HLA-C (table 6):

5'CGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 132)
5'TCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 133)
5'GTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 134)
5'GGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 135)
5'GGGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 136)

5'CGGGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 137)

5'CGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 138)
5'TCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 139)
5'CTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 140)
5'CCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 141)
5'CCCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 142)
5'ACCCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 143)

In another preferred embodiment, the present invention relates to any method as described above, further characterized that:

- the amplification of exon 2 is carried out with at least one of the following forward primers:
    - for HLA-A: 5APBio (SEQ ID NO 144);
    - for HLA-B: IBPin1 (SEQ ID NO 145);
    - for HLA-C: 5CIN1 (SEQ ID NO 146);
- the amplification of exon 3 is carried out with at least one of the following reverse primers:
    - for HLA-A: 3APBio (SEQ ID NO 147);
    - for HLA-B: IBPin3 (SEQ ID NO 148);
    - for HLA-C: 3CIN3 (SEQ ID NO 149).

The skilled man will understand that these primers (SEQ ID NOs 1 to 149) may be adapted by addition or deletion of one or more nucleotides at their extremities. Such adaptations may be required, for instance, if the conditions of amplification are changed, if the amplified material is RNA instead of DNA, as is the case, for example, in the NASBA system.

The present invention further relates to a primer as described above, for use in the amplification of exon 2 of HLA-A, HLA-B or HLA-C alleles, said primer specifically

hybridizing to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C; more specifically, said primer specifically hybridizing to the locus-specific target sequences situated at positions:

- 67, 96, 109, 110, 111, 118, 123, 131 or 181 of the HLA-A intron 2; or
- 35 or 170 of the HLA-B intron 2; or
- 96, 119 or 154 of the HLA-C intron 2;

more specifically, said primer being chosen from table 1 (for amplification of exon 2 of HLA-A), from table 3 (for amplification of exon 2 of HLA-B) or from table 5 (for amplification of exon 2 of HLA-C).

The present invention further also relates to a primer as described above, for use in the amplification of exon 3 of HLA-A, HLA-B or HLA-C alleles said primer specifically hybridizing to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C; more specifically, said primer specifically hybridizing to the locus-specific target sequences situated at positions:

- 67, 96, 109, 110, 111, 118, 123, 131 or 181 of the HLA-A intron 2; or
- 35 or 170 of the HLA-B intron 2; or
- 96, 119 or 154 of the HLA-C intron 2;

more specifically, said primer being chosen from table 2 (for amplification of exon 3 of HLA-A), from table 4 (for amplification of exon 3 of HLA-B) or from table 6 (for amplification of exon 3 of HLA-C).

In another preferred embodiment, the present invention further relates to a primer set consisting of a combination of a forward and a reverse primer as defined above, for use in the amplification of exon 2 of HLA-A, HLA-B or HLA-C alleles. In a particularly preferred embodiment, the present invention relates to the combination of the forward primer 5APBio (SEQ ID NO 144) for HLA-A, IBPin1 (SEQ ID NO 145)

for HLA-B or 5CIN1 (SEQ ID NO 146) for HLA-C and a reverse primer specifically hybridizing to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C; more specifically, said reverse primer specifically hybridizing to the locus-specific target sequences situated at positions:

- 67, 96, 109, 110, 111, 118, 123, 131 or 181 of the HLA-A intron 2; or
- 35 or 170 of the HLA-B intron 2; or
- 96, 119 or 154 of the HLA-C intron 2;

more specifically, said reverse primer being chosen from table 1 (for amplification of exon 2 of HLA-A), from table 3 (for amplification of exon 2 of HLA-B) or from table 5 (for amplification of exon 2 of HLA-C).

In another preferred embodiment, the present invention relates to a primer set consisting of a combination of a forward and a reverse primer as described above, for use in the amplification of exon 3 of HLA-A, HLA-B or HLA-C alleles. In a particularly preferred embodiment, the present invention relates to the combination of a forward primer specifically hybridizing to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C; more specifically, said forward primer specifically hybridizing to the locus-specific target sequences situated at positions:

- 67, 96, 109, 110, 111, 118, 123, 131 or 181 of the HLA-A intron 2; or
- 35 or 170 of the HLA-B intron 2; or
- 96, 119 or 154 of the HLA-C intron 2;

more specifically, said forward primer being chosen from table 2 (for amplification of exon 3 of HLA-A), from table 4 (for amplification of exon 3 of HLA-B) or from table 6 (for amplification of exon 3 of HLA-C) and the reverse primer 3APBio (SEQ ID NO 147) for HLA-A, IBPin3 (SEQ ID NO 148) for HLA-B or 3CIN3 (SEQ ID NO 149 for HLA-C.

Most preferably, the primers are used in a mix. Accordingly, the present invention relates to a multiplex primer mix containing at least one primer pair as described above for amplification of exon 2 and one primer pair as described above for the amplification of exon 3.

The primers of the invention may be labeled. Labeling may be carried out by any method known to the person skilled in the art. The nature of the label may be isotopic (^{32}P, ^{35}S, etc.) or non-isotopic (biotin, digoxigenin, etc.).

The oligonucleotides used as primers may also comprise nucleotide analogues such as phosphorothiates (Matsukura et al., 1987), alkylphosphorothiates (Miller et al., 1979) or peptide nucleic acids (Nielsen et al., 1991; Nielsen et al., 1993) or may contain intercalating agents (Asseline et al., 1984). As most other variations or modifications introduced into the original DNA sequences of the invention these variations will necessitate adaptions with respect to the conditions under which the oligonucleotide should be used to obtain the required specificity and sensitivity. However the eventual results of hybridization will be essentially the same as those obtained with the unmodified oligonucleotides. The introduction of these modifications may be advantageous in order to positively influence characteristics such as hybridization kinetics, reversibility of the hybrid-formation, biological stability of the oligonucleotide molecules, etc.

The present invention also relates to a method for typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following steps:

(i) if needed, release, isolation and/or concentration of the nucleic acids present in said sample;

(ii) amplification of the nucleic acids according to the invention;

(iii) typing of the specific HLA-A, HLA-B or HLA-C alleles present in said sample.

Release, concentration and isolation of the nucleic acids from the sample can be done by any method known in the art. Currently, various commercial kits are available such as the QIAamp Blood Kit from Qiagen (Hilden, Germany) for the isolation of nucleic acids from blood samples. The nucleic acids are subsequently amplified by the method of the invention described above. The products of this amplification step are then ideally suited for typing of the specific allele present in the sample. Currently 133 different alleles of HLA-A, 274 different alleles of HLA-B and 77 different alleles of HLA-C are known. Typing of these alleles can be done by any method known in the art, such as duplex analysis of the PCR products (Clay et al., 1994), single-stranded conformational polymorphism analysis of the PCR product (PCR-SSCP; Yoshida *et al.*, 1992), sequence-based typing (SBT; Santamaria et al., 1992 and 1993), the use of sequence specific primers in PCR reaction (PCR-SSP; Olerup and Zetterquist, 1991), the use of PCR in combination with sequence-specific oligonucleotide probing (PCR-SSOP; Saiki et al., 1986), conventional dot-blot, Southern blot, sandwich or probing by reverse dot-blot (Saiki et al., 1989). However, in order to obtain fast and easy results if a multitude of probes is involved, a reverse hybridization format may be convenient. Accordingly, in a preferred embodiment the selected probes are immobilized to certain locations on a solid support and the amplified polynucleic acids are labeled in order to enable the detection of the hybrids formed. The term "solid support" can refer to any substrate to which an oligonucleotide probe can be coupled, provided that it retains its hybridization characteristics and provided that the background level of hybridization remains low. Usually the solid substrate will be a microtiter plate (e.g. in the DEIA technique), a

membrane (e.g. nylon or nitrocellulose) or a microsphere (bead) or a chip. Prior to application to the membrane or fixation it may be convenient to modify the nucleic acid probe in order to facilitate fixation or improve the hybridization efficiency. Such modifications may encompass homopolymer tailing, coupling with different reactive groups such as aliphatic groups, NH_2 groups, SH groups, carboxylic groups, or coupling with biotin, haptens or proteins.

The present invention further relates to a diagnostic kit for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following components:

(i) when appropriate, a means for releasing, isolating or concentrating the nucleic acids present in said sample;

(ii) a primer set or a primer mix according to the invention;

(iv) a means for typing of the specific HLA-A, HLA-B or HLA-C alleles present in said sample.

A specific and very user-friendly diagnostic kit is the a line probe assay for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following components:

(i) when appropriate, a means for releasing, isolating or concentrating the nucleic acids present in said sample;

(ii) a primer pair or a primer mix according to the invention;

(iii) at least one probe that specifically hybridizes with one of the HLA-A, HLA-B or HLA-C alleles, fixed to a solid support;

(iv) a hybridization buffer, or components necessary for producing said buffer;

(v) a wash solution, or components necessary for producing said solution;

(vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

In this embodiment, the selected set of probes is immobilized to a membrane strip in a line fashion. Said probes may be immobilized individually or as mixtures to the delineated locations. The amplified HLA-A, HLA-B or HLA-C polynucleic acids can be labelled with biotine, and the hybrid can then, via a biotine-streptavidine coupling, be detected with a non-radioactive colour developing system.

The term "hybridization buffer" means a buffer allowing a hybridization reaction between the probes and the polynucleic acids present in the sample, or the amplified products, under the appropriate stringency conditions.

The term "wash solution" means a solution enabling washing of the hybrids formed under the appropriate stringency conditions.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of stated integers or steps but not to the exclusion of any other integer or step or group of integers or steps.

Figure legends

Figure 1. Alignment of 29 HLA-A intron 2 sequences. The consensus sequence is shown on top of the figure. Nucleotides conform to the consensus sequence are indicated with a vertical line. Nucleotides that differ from the consensus sequence are indicated. Sequencing was carried out according to example 1.

Figure 2. Alignment of 38 HLA-B intron 2 sequences. The consensus sequence is shown on top of the figure. Nucleotides conform to the consensus sequence are indicated with a vertical line. Nucleotides that differ from the consensus sequence are indicated. Sequencing was carried out according to example 1.

Figure 3. Alignment of 13 HLA-C intron 2 sequences. The consensus sequence is shown on top of the figure. Nucleotides conform to the consensus sequence are indicated with a vertical line. Nucleotides that differ from the consensus sequence are indicated. Sequencing was carried out according to example 1.

Tables

Table 1. Primers used for the amplification of HLA-A exon 2.

Position in intron 2	Sequence[1]	SEQ ID NO
67	ATCTCGGACCCGGAGACTGT	1
	GATCTCGGACCCGGAGACTGT	2
	GGATCTCGGACCCGGAGACTGT	3
	YGGATCTCGGACCCGGAGACTGT	4
	GYGGATCTCGGACCCGGAGACTGT	5
	GGYGGATCTCGGACCCGGAGACTGT	6
96	GGTCTCGGRGTCCCGCGGCT	7
	GGGTCTCGGRGTCCCGCGGCT	8
	AGGGTCTCGGRGTCCCGCGGCT	9
	AAGGGTCTCGGRGTCCCGCGGCT	10
	CAAGGGTCTCGGRGTCCCGCGGCT	11
109	CTCCCGGGDCAAGGGTCTCG	12
	TCTCCCGGGDCAAGGGTCTCG	13
	CTCTCCCGGGDCAAGGGTCTCG	14
	GCCTCTCCCGGGDCAAGGGTCTCG	15
	GCCTCTCCCGGGDCAAGGGTCTCG	16
	GGCCTCTCCCGGGDCAAGGGTCTCG	17
110	TCTCCCGGGDCAAGGGTCTC	18
	CTCTCCCGGGDCAAGGGTCTC	19
	CCTCTCCCGGGDCAAGGGTCTC	20
	GCCTCTCCCGGGDCAAGGGTCTC	21
	GGCCTCTCCCGGGDCAAGGGTCTC	22
	GGGCCTCTCCCGGGDCAAGGGTCTC	23
111	CTCTCCCGGGDCAAGGGTCT	24
	CCTCTCCCGGGDCAAGGGTCT	25
	GCCTCTCCCGGGDCAAGGGTCT	26
	GGCCTCTCCCGGGDCAAGGGTCT	27

	GGGCCTCTCCCGGGDCAAGGGTCT	28
	TGGGCCTCTCCCGGGDCAAGGGTCT	29
118	CCTGGGCCTCTCCCGGGDCA	30
	GCCTGGGCCTCTCCCGGGDCA	31
	CGCCTGGGCCTCTCCCGGGDCA	32
	GCGCCTGGGCCTCTCCCGGGDCA	33
	GGCGCCTGGGCCTCTCCCGGGDCA	34
	AGGCGCCTGGGCCTCTCCCGGGDCA	35
123	AGGCGCCTGGGCCTCTCCCG	36
	AAGGCGCCTGGGCCTCTCCCG	37
	WAAGGCGCCTGGGCCTCTCCCG	38
	TWAAGGCGCCTGGGCCTCTCCCG	39
	GTWAAGGCGCCTGGGCCTCTCCCG	40
	GGTWAAGGCGCCTGGGCCTCTCCCG	41
131	CCGGGTWAAGGCGCCTGGGC	42
	ACCGGGTWAAGGCGCCTGGGC	43
	AACCGGGTWAAGGCGCCTGGGC	44
	AAACCGGGTWAAGGCGCCTGGGC	45
	GAAACCGGGTWAAGGCGCCTGGGC	46
	TGAAACCGGGTWAAGGCGCCTGGGC	47
181	YCCVGCCCCGACCAACCYGG	48
	GYCCVGCCCCGACCAACCYGG	49
	YGYCCVGCCCCGACCAACCYGG	50
	CYGYCCVGCCCCGACCAACCYGG	51
	CCYGYCCVGCCCCGACCAACCYGG	52
	CCCYGYCCVGCCCCGACCAACCYGG	53

[1]All sequences are given from 5' to 3'.

Table 2. Primers used for the amplification of HLA-A exon 3.

Position in intron 2	Sequence[1]	SEQ ID NO
67	CGGACGGGCCRGGTSRCCCA	54
	ACGGACGGGCCRGGTSRCCCA	55
	CACGGACGGGCCRGGTSRCCCA	56
	CCACGGACGGGCCRGGTSRCCCA	57
	CCCACGGACGGGCCRGGTSRCCCA	58
	CCCCACGGACGGGCCRGGTSRCCCA	59
96	GGTCCGAGATCCRCCCCGAA	60
	GGGTCCGAGATCCRCCCCGAA	61
	CGGGTCCGAGATCCRCCCCGAA	62
	CCGGGTCCGAGATCCRCCCCGAA	63
	TCCGGGTCCGAGATCCRCCCCGAA	64
	CTCCGGGTCCGAGATCCRCCCCGAA	65
109	CCCCGAAGCCGCGGGACYCC	66
	RCCCCGAAGCCGCGGGACYCC	67
	CRCCCCGAAGCCGCGGGACYCC	68
	CCRCCCCGAAGCCGCGGGACYCC	69
	TCCRCCCCGAAGCCGCGGGACYCC	70
	ATCCRCCCCGAAGCCGCGGGACYCC	71
110	CCCGAAGCCGCGGGACYCCG	72
	CCCCGAAGCCGCGGGACYCCG	73
	RCCCCGAAGCCGCGGGACYCCG	74
	CRCCCCGAAGCCGCGGGACYCCG	75
	CCRCCCCGAAGCCGCGGGACYCCG	76
	TCCRCCCCGAAGCCGCGGGACYCCG	77
111	CCGAAGCCGCGGGACYCCGA	78
	CCCGAAGCCGCGGGACYCCGA	79
	CCCCGAAGCCGCGGGACYCCGA	80
	RCCCCGAAGCCGCGGGACYCCGA	81
	CRCCCCGAAGCCGCGGGACYCCGA	82

	CCRCCCCGAAGCCGCGGGACYCCGA	83
118	CGCGGGACYCCGAGACCCTT	84
	CCGCGGGACYCCGAGACCCTT	85
	GCCGCGGGACYCCGAGACCCTT	86
	AGCCGCGGGACYCCGAGACCCTT	87
	AAGCCGCGGGACYCCGAGACCCTT	88
	GAAGCCGCGGGACYCCGAGACCCTT	89
123	GACYCCGAGACCCTTGDCCC	90
	GGACYCCGAGACCCTTGDCCC	91
	GGGACYCCGAGACCCTTGDCCC	92
	CGGGACYCCGAGACCCTTGDCCC	93
	GCGGGACYCCGAGACCCTTGDCCC	94
	CGCGGGACYCCGAGACCCTTGDCCC	95
131	GACCCTTGDCCCGGGAGAGG	96
	AGACCCTTGDCCCGGGAGAGG	97
	GAGACCCTTGDCCCGGGAGAGG	98
	CGAGACCCTTGDCCCGGGAGAGG	99
	CCGAGACCCTTGDCCCGGGAGAGG	100
	YCCGAGACCCTTGDCCCGGGAGAGG	101
181	GTTTAGGCCAAAAATCCCCC	102
	AGTTTAGGCCAAAAATCCCCC	103
	CAGTTTAGGCCAAAAATCCCCC	104
	TCAGTTTAGGCCAAAAATCCCCC	105
	TTCAGTTTAGGCCAAAAATCCCCC	106
	TTTCAGTTTAGGCCAAAAATCCCCC	107

[1]All sequences are given from 5' to 3'.

Table 3. Primers used for the amplification of HLA-B exon 2.

Position in intron 2	Sequence[1]	SEQ ID NO
170	CCCGCGGGGATTTTTGGCCTC	108
	ACCCGCGGGGATTTTTGGCCTC	109
	AACCCGCGGGGATTTTTGGCCTC	110
	CAACCCGCGGGGATTTTTGGCCTC	111
	CCAACCCGCGGGGATTTTTGGCCTC	112
	MCCAACCCGCGGGGATTTTTGGCCTC	113

[1]All sequences are given from 5' to 3'.

Table 4. Primers used for the amplification of HLA-B exon 3.

Position in intron 2	Sequence[1]	SEQ ID NO
35	CYGGGGCGSAGGTCACGACT	114
	CCYGGGGCGSAGGTCACGACT	115
	GCCYGGGGCGSAGGTCACGACT	116
	GGCCYGGGGCGSAGGTCACGACT	117
	CGGCCYGGGGCGSAGGTCACGACT	118
	CCGGCCYGGGGCGSAGGTCACGACT	119
170	CCCGGTTTCATTTTCAGTTG	120
	ACCCGGTTTCATTTTCAGTTG	121
	TACCCGGTTTCATTTTCAGTTG	122
	YTACCCGGTTTCATTTTCAGTTG	123
	TYTACCCGGTTTCATTTTCAGTTG	124
	STYTACCCGGTTTCATTTTCAGTTG	125

[1]All sequences are given from 5' to 3'.

Table 5. Primers used for the amplification of HLA-C exon 2.

Position in intron 2	Sequence[1]	SEQ ID NO
119	GTCGAGGGTCTGGGCGGGTT	126
	GGTCGAGGGTCTGGGCGGGTT	127
	CGGTCGAGGGTCTGGGCGGGTT	128
	CCGGTCGAGGGTCTGGGCGGGTT	129
	YCCGGTCGAGGGTCTGGGCGGGTT	130
	CYCCGGTCGAGGGTCTGGGCGGGTT	131

[1]All sequences are given from 5' to 3'.

Table 6. Primers used for the amplification of HLA-C exon 3.

Position in intron 2	Sequence[1]	SEQ ID NO
96	CGCCCCRAGTCTCCSSGTCT	132
	TCGCCCCRAGTCTCCSSGTCT	133
	GTCGCCCCRAGTCTCCSSGTCT	134
	GGTCGCCCCRAGTCTCCSSGTCT	135
	GGGTCGCCCCRAGTCTCCSSGTCT	136
	CGGGTCGCCCCRAGTCTCCSSGTCT	137
154	CGRCCGGRGAGAGCCCCAGT	138
	TCGRCCGGRGAGAGCCCCAGT	139
	CTCGRCCGGRGAGAGCCCCAGT	140
	CCTCGRCCGGRGAGAGCCCCAGT	141
	CCCTCGRCCGGRGAGAGCCCCAGT	142
	ACCCTCGRCCGGRGAGAGCCCCAGT	143

[1]All sequences are given from 5' to 3'.

Examples

Example 1: Sequence determination of intron 2 of various HLA-A, HLA-B and HLA-C alleles

Nucleic acids were prepared from different blood samples by use of the QIAamp Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Part of exon 1, intron 2, exon 2, intron 2 and exon 3 of HLA-A was amplified by use of the following primer set: 5APBio (SEQ ID NO 144) as forward primer;

3APBio (SEQ ID NO 147) as reverse primer.

Exon 2, intron 2 and exon 3 of HLA-B was amplified by use of the following primer set: IBPin1 (SEQ ID NO 145) as forward primer;

IBPin3 (SEQ ID NO 148) as reverse primer;

Exon 2, intron 2 and exon 3 of HLA-C was amplified by use of the following primer set: 5CIN1 (SEQ ID NO 146) as forward primer;

3CIN3 (SEQ ID NO149) as reverse primer;

The PCR reaction cycle was composed of the following steps:

1 min at 96°C

5 times (30 s at 95°C; 50 s at 64°C; 50 s at 72°C)

5 times (30 s at 95°C; 50 s at 62°C; 50 s at 72°C)

10 times (30 s at 95°C; 50 s at 60°C; 50 s at 72°C)

15 times (30 s at 95°C; 50 s at 55°C; 50 s at 72°C)

10 min at 72°C

The amplification reaction was carried out in 50 mM Tris-HCl pH 9.2, 16 mM $(NH_4)_2SO_4$, 200 µM dNTPs, 2.5 U Taq polymerase, 1.5 mM $MgCl_2$, 15 pmole of each primer and 0.1 to 0.5 µg DNA.

The resulting amplicon was cloned in the pGEMt-vector (Promega, Madison, WI, USA). Nucleotide sequence analysis was performed by use of an automated DNA sequencer Model 373A (Applied Biosystems, Foster City, CA, USA) with fluorescence-labelled dideoxy nucleotides (PrismTM Ready Reaction Dye Terminator Cycle Sequencing Kit; Applied Biosystems, Foster City, CA, USA). The primers used for the sequencing reaction were the same as for the amplification step. Eighteen intron 2 sequences were obtained for HLA-A, 51 for HLA-B and 20 for HLA-C. The sequences are shown in figures 1, 2 and 3, respectively.

Example 2: Amplification of exon 2 and exon 3 of HLA-A

Nucleic acids were prepared from different blood samples by use of the QIAamp Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Based on the sequence alignment of the HLA-A intron 2 sequences (figure 1), a reverse and a forward, locus-specific primer was designed for the specific amplification of the HLA-A exon 2 and exon 3, respectively. With these primers, a primer mix was constructed for the separate amplification of exon 2 and exon 3 of HLA-A consisting of the following 2 primer sets:

- for exon 2: 5APBio (SEQ ID NO 144) as forward primer and 5'ATCTCGGACCCGGAGACTGT3' (SEQ ID NO 1) as reverse primer;
- for exon 3: 5'CAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 104) as forward primer and 3APBio (SEQ ID NO 147) as reverse primer.

The PCR reaction cycle was composed of the following steps:

5 min at 96°C

35 times (30 s at 96°C; 20 s at 58°C; 30 s at 72°C)

10 min at 72°C.

The PCR reaction was carried out in 10 mM Tris.HCl pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 0.001% (w/v) gelatine; 200µM dATP, dGTP, dCTP, dTTP (final concentrations) and 20 pmol of each primer and 1U AmpliTaq (Applied Biosystems, Foster City, CA, USA). The length of the obtained PCR products was verified on an agarose gel according to Sambrook et al. (1989).

Example 3: Amplification of exon 2 and exon 3 of HLA-B

Nucleic acids were prepared from different blood samples by use of the QIAamp Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Based on the sequence alignment of the HLA-B intron 2 sequences (figure 2), a reverse and a forward, locus-specific primer was designed for the specific amplification of the HLA-B exon 2 and exon 3, respectively. With these primers, a primer mix was constructed for the separate amplification of exon 2 and exon 3 of HLA-B consisting of the following 2 primer sets:

- for exon 2: IBPin1 (SEQ ID NO 145) as forward primer and 5'ACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 109) as reverse primer;
- for exon 3: 5'ACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 121) as forward primer and IBPin3 (SEQ ID NO 148) as reverse primer.

The PCR reaction cycle was composed of the following steps:

5 min at 96°C

35 times (30 s at 96°C; 20 s at 58°C; 30 s at 72°C)

10 min at 72°C.

The PCR reaction was carried out in 10 mM Tris.HCl pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 0.001% (w/v) gelatine; 200µM dATP, dGTP, dCTP, dTTP (final concentrations) and 20 pmol of each primer and 1U AmpliTaq (Applied Biosystems,

Foster City, CA, USA). The length of the obtained PCR products was verified on an agarose gel according to Sambrook et al. (1989).

Example 4: Amplification of exon 2 and exon 3 of HLA-C

Nucleic acids were prepared from different blood samples by use of the QIAamp Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Based on the sequence alignment of the HLA-C intron 2 sequences (figure 3), a reverse and a forward, locus-specific primer was designed for the specific amplification of the HLA-C exon 2 and exon 3, respectively. With these primers, a primer mix was constructed for the separate amplification of exon 2 and exon 3 of HLA-C consisting of the following 2 primer sets:

- for exon 2: 5CIN1 (SEQ ID NO 146) as forward primer and 5'GGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 127) as reverse primer;
- for exon 3: 5'TCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 139) as forward primer and 3CIN3 (SEQ ID NO 149) as reverse primer.

The PCR reaction cycle was composed of the following steps:

5 min at 96°C

35 times (30 s at 96°C; 20 s at 58°C; 30 s at 72°C)

10 min at 72°C.

The PCR reaction was carried out in 10 mM Tris.HCl pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 0.001% (w/v) gelatine; 200µM dATP, dGTP, dCTP, dTTP (final concentrations) and 20 pmol of each primer and 1U AmpliTaq (Applied Biosystems, Foster City, CA, USA). The length of the obtained PCR products was verified on an agarose gel according to Sambrook et al. (1989).

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Claims

1. Method for the locus-specific, separate amplification of exon 2 and/or exon 3 of HLA-A, HLA-B or HLA-C alleles, making use of at least one primer set wherein:
   - for the amplification of exon 2, the reverse primer specifically hybridizes to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C.
   - for the amplification of exon 3, the forward primer specifically hybridizes to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C.

2. Method according to claim 1 further characterized that the locus-specific target sequences are situated at positions:
   - 67, 96, 109, 110, 111, 118, 123, 131 or 181 of the HLA-A intron 2; or
   - 35 or 170 of the HLA-B intron 2; or
   - 96, 119 or 154 of the HLA-C intron 2.

3. Method according to claim 2 further characterized that said positions constitute the 3' end of the primer that is used for the amplification of exon 2 or exon 3.

4. Method according to claim 3 further characterized that the primer is chosen from the following list:
   - for the amplification of exon 2 of HLA-A (table 1):

     5'ATCTCGGACCCGGAGACTGT3' (SEQ ID NO 1)
     5'GATCTCGGACCCGGAGACTGT3' (SEQ ID NO 2)
     5'GGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 3)

5'YGGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 4)
5'GYGGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 5)
5'GGYGGATCTCGGACCCGGAGACTGT3' (SEQ ID NO 6)

5'GGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 7)
5'GGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 8)
5'AGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 9)
5'AAGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 10)
5'CAAGGGTCTCGGRGTCCCGCGGCT3' (SEQ ID NO 11)

5'CTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 12)
5'TCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 13)
5'CTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 14)
5'CCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 15)
5'GCCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 16)
5'GGCCTCTCCCGGGDCAAGGGTCTCG3' (SEQ ID NO 17)

5'TCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 18)
5'CTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 19)
5'CCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 20)
5'GCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 21)
5'GGCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 22)
5'GGGCCTCTCCCGGGDCAAGGGTCTC3' (SEQ ID NO 23)

5'CTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 24)
5'CCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 25)
5'GCCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 26)
5'GGCCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 27)
5'GGGCCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 28)
5'TGGGCCTCTCCCGGGDCAAGGGTCT3' (SEQ ID NO 29)

5'CCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 30)
5'GCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 31)
5'CGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 32)
5'GCGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 33)
5'GGCGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 34)
5'AGGCGCCTGGGCCTCTCCCGGGDCA3' (SEQ ID NO 35)

5'AGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 36)
5'AAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 37)
5'WAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 38)
5'TWAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 39)
5'GTWAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 40)
5'GGTWAAGGCGCCTGGGCCTCTCCCG3' (SEQ ID NO 41)

5'CCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 42)
5'ACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 43)
5'AACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 44)
5'AAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 45)
5'GAAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 46)
5'TGAAACCGGGTWAAGGCGCCTGGGC3' (SEQ ID NO 47)

5'YCCVGCCCCGACCAACCYGG3' (SEQ ID NO 48)
5'GYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 49)
5'YGYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 50)
5'CYGYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 51)
5'CCYGYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 52)
5'CCCYGYCCVGCCCCGACCAACCYGG3' (SEQ ID NO 53)

- for the amplification of exon 3 of HLA-A (table 2):

5'CGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 54)
5'ACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 55)
5'CACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 56)
5'CCACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 57)
5'CCCACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 58)
5'CCCCACGGACGGGCCRGGTSRCCCA3' (SEQ ID NO 59)

5'GGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 60)
5'GGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 61)
5'CGGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 62)
5'CCGGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 63)
5'TCCGGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 64)
5'CTCCGGGTCCGAGATCCRCCCCGAA3' (SEQ ID NO 65)

5'CCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 66)

5'RCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 67)
5'CRCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 68)
5'CCRCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 69)
5'TCCRCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 70)
5'ATCCRCCCCGAAGCCGCGGGACYCC3' (SEQ ID NO 71)

5'CCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 72)
5'CCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 73)
5'RCCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 74)
5'CRCCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 75)
5'CCRCCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 76)
5'TCCRCCCCGAAGCCGCGGGACYCCG3' (SEQ ID NO 77)

5'CCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 78)
5'CCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 79)
5'CCCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 80)
5'RCCCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 81)
5'CRCCCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 82)
5'CCRCCCCGAAGCCGCGGGACYCCGA3' (SEQ ID NO 83)

5'CGCGGGACYCCGAGACCCTT3' (SEQ ID NO 84)
5'CCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 85)
5'GCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 86)
5'AGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 87)
5'AAGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 88)
5'GAAGCCGCGGGACYCCGAGACCCTT3' (SEQ ID NO 89)

5'GACYCCGAGACCCTTGDCCC3' (SEQ ID NO 90)
5'GGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 91)
5'GGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 92)
5'CGGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 93)
5'GCGGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 94)
5'CGCGGGACYCCGAGACCCTTGDCCC3' (SEQ ID NO 95)

5'GACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 96)
5'AGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 97)
5'GAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 98)

5'CGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 99)
5'CCGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 100)
5'YCCGAGACCCTTGDCCCGGGAGAGG3' (SEQ ID NO 101)

5'GTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 102)
5'AGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 103)
5'CAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 104)
5'TCAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 105)
5'TTCAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 106)
5'TTTCAGTTTAGGCCAAAAATCCCCC3' (SEQ ID NO 107)

- for the amplification of exon 2 of HLA-B (table 3):

5'CCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 108)
5'ACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 109)
5'AACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 110)
5'CAACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 111)
5'CCAACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 112)
5'MCCAACCCGCGGGGATTTTTGGCCTC3' (SEQ ID NO 113)

- for the amplification of exon 3 of HLA-B (table 4):

5'CYGGGGCGSAGGTCACGACT3' (SEQ ID NO 114)
5'CCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 115)
5'GCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 116)
5'GGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 117)
5'CGGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 118)
5'CCGGCCYGGGGCGSAGGTCACGACT3' (SEQ ID NO 119)

5'CCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 120)
5'ACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 121)
5'TACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 122)
5'YTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 123)
5'TYTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 124)
5'STYTACCCGGTTTCATTTTCAGTTG3' (SEQ ID NO 125)

- for the amplification of exon 2 of HLA-C (table 5):

5'GTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 126)

5'GGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 127)
5'CGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 128)
5'CCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 129)
5'YCCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 130)
5'CYCCGGTCGAGGGTCTGGGCGGGTT3' (SEQ ID NO 131)

- for the amplification of exon 3 of HLA-C (table 6):

5'CGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 132)
5'TCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 133)
5'GTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 134)
5'GGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 135)
5'GGGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 136)
5'CGGGTCGCCCCRAGTCTCCSSGTCT3' (SEQ ID NO 137)

5'CGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 138)
5'TCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 139)
5'CTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 140)
5'CCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 141)
5'CCCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 142)
5'ACCCTCGRCCGGRGAGAGCCCCAGT3' (SEQ ID NO 143)

5. Method according to any of claims 1 to 4 further characterized that:

- the amplification of exon 2 is carried out with at least one of the following forward primers:
    - for HLA-A: 5APBio (SEQ ID NO 144); or
    - for HLA-B: IBPin1 (SEQ ID NO 145); or
    - for HLA-C: 5CIN1 (SEQ ID NO 146);
- the amplification of exon 3 is carried out with at least one of the following reverse primers:

for HLA-A: 3APBio (SEQ ID NO 147); or

for HLA-B: IBPin3 (SEQ ID NO 148); or

for HLA-C: 3CIN3 (SEQ ID NO 149).

6. Method according to any of claims 1 to 5 further characterized that both exon 2 and exon 3 of HLA-A, HLA-B or HLA-C are amplified by use of a multiplex primer mix containing at least one primer pair for amplification of exon 2 and at least one primer pair for amplification of exon 3.

7. A primer as defined by any of claims 1 to 4, for use in the amplification of exon 2 of HLA-A, HLA-B or HLA-C alleles.

8. A primer as defined by any of claims 1 to 4, for use in the amplification of exon 3 of HLA-A, HLA-B or HLA-C alleles.

9. A primer set consisting of a combination of a forward and a reverse primer as defined in any of claims 1 to 5, for use in the amplification of exon 2 of HLA-A, HLA-B or HLA-C alleles.

10. A primer set consisting of a combination of a forward and a reverse primer as defined in any of claims 1 to 5, for use in the amplification of exon 3 of HLA-A, HLA-B or HLA-C alleles.

11. A multiplex primer mix containing at least one primer pair according to claim 9 for amplification of exon 2 and one primer pair according to claim 10 for amplification of exon 3.

12. Method for typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following steps:

(v) if needed, release, isolation and/or concentration of the nucleic acids present in said sample;

(vi) amplification of the nucleic acids according to any of claims 1 to 6;

(vii) typing of the specific HLA-A, HLA-B or HLA-C allele present in said sample.

13. Method according to claim 12 further characterized that the typing step is carried out by hybridization with one or more suitable probes.

14. A diagnostic kit for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following components:

(vii) when appropriate, a means for releasing, isolating or concentrating the nucleic acids present in said sample;

(viii) a primer set or a primer mix according to any of claims 7-11

(viii) a means for typing of the specific HLA-A, HLA-B and/or HLA-C allele present in said sample.

15. A line probe assay for the typing or subtyping of one or more HLA-A, HLA-B or HLA-C alleles in a sample comprising the following components:

(iii) when appropriate, a means for releasing, isolating or concentrating the nucleic acids present in said sample;

(iv) a primer pair or a primer mix according to any of claims 7-11;

(ix) at least one probe that specifically hybridizes with exon 2 or exon 3 of HLA-A, HLA-B or HLA-C, fixed to a solid support;

(x) a hybridization buffer, or components necessary for producing said buffer;

(xi) a wash solution, or components necessary for producing said solution;

(xii) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

Abstract

The present invention relates to a method and to specific primers for the locus-specific, separate amplification of exon 2 and/or exon 3 of HLA-A, HLA-B or HLA-C alleles, making use of at least one primer set wherein:

- for the amplification of exon 2, the reverse primer specifically hybridizes to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C.
- for the amplification of exon 3, the forward primer specifically hybridizes to a locus-specific target sequence in intron 2 of respectively HLA-A, HLA-B or HLA-C.

In accordance, the present invention provides a method for the typing or subtyping of HLA Class I alleles making use of the amplification method of the invention.

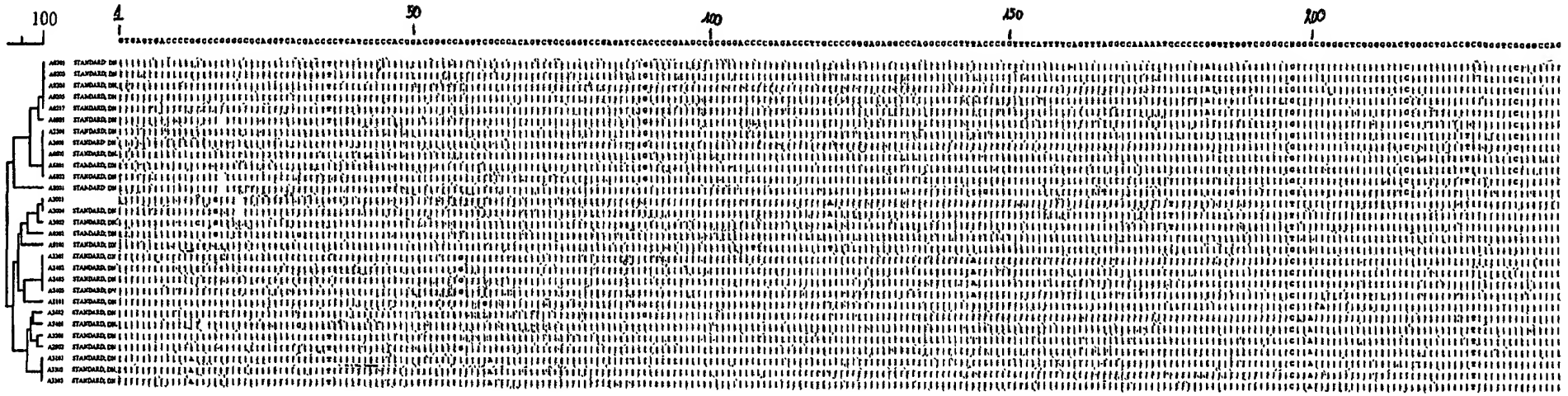

Figure 1

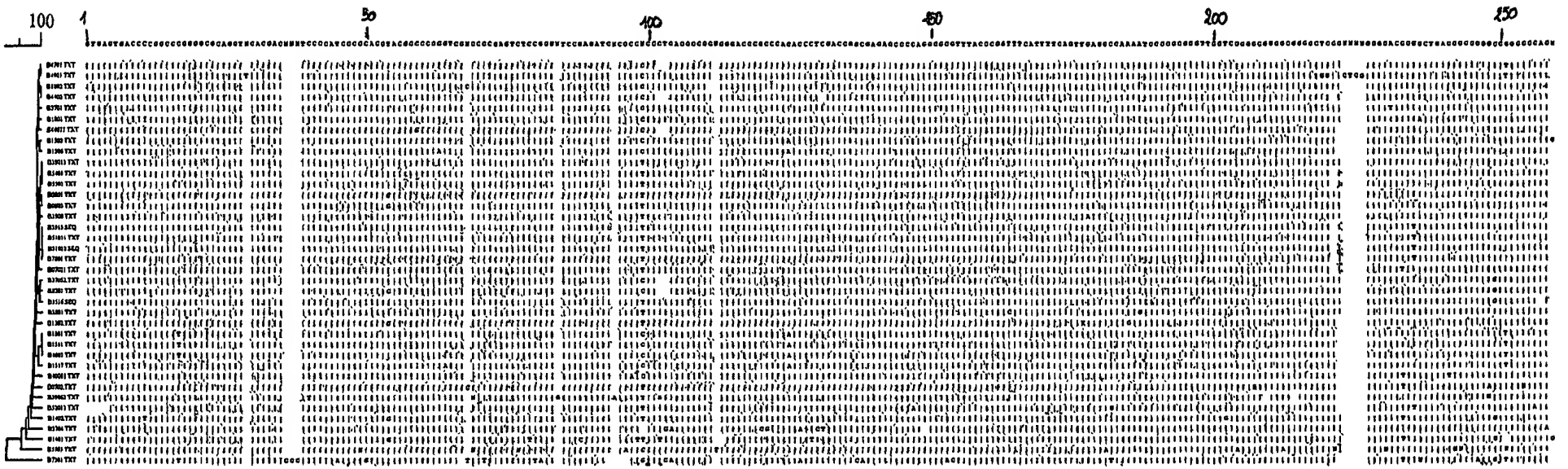

Figure 2

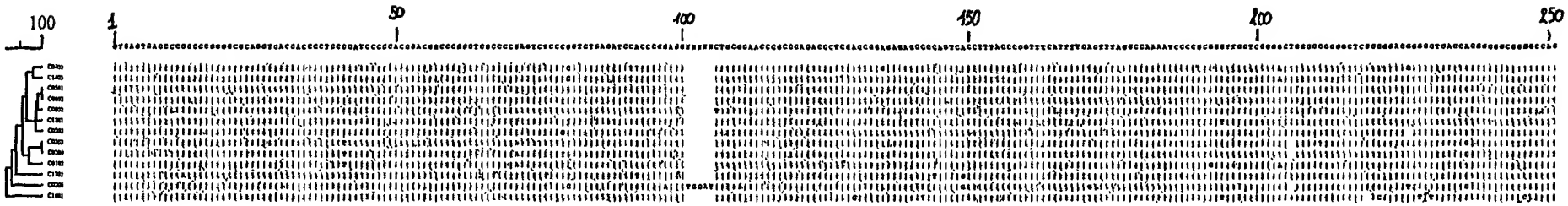

Figure 3